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Pellaea flavescens, a Brazilian Endemic, is a Synonym of Old World *Pellaea viridis*

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Abstract.—A phylogenetic analysis of plastid *rbcL* sequences indicates that *Pellaea flavescens* from Brazil is nested within the Old World species *Pellaea viridis*. The morphology of these two species is similar and *P. flavescens* is here considered to be a synonym of the older *P. viridis*. This fern may have been introduced to and subsequently naturalized in Brazil or, alternatively, the natural result of long distance dispersal.

Key Words.—Pteridaceae, cheilanthoid, *rbcL*, phylogeny, ferns

Pellaea flavescens Fée, described from Rio de Janeiro and recently found in São Paulo, Brazil (Prado and Hirai, 2011), is morphologically similar to *Pellaea viridis* (Forssk.) Prantl from Africa, Madagascar, the Comoros, and the Mascarenes (Moran and Smith, 2001). Nonetheless, Tryon and Tryon (1982) placed *P. flavescens* in *Pellaea* section *Ormopteris*, a small group of species mostly endemic to central Brazil, while referring *P. viridis* to *Cheilanthes*. Several recent phylogenetic analyses (Gastony and Rollo, 1995; Kirkpatrick, 2007; Prado *et al.*, 2007; Schuettpelz *et al.*, 2007; Eiserhardt *et al.*, 2011) have demonstrated that both *Pellaea* and *Cheilanthes*, as defined by Tryon and Tryon (1982), are polyphyletic. These studies also showed that *P. viridis* and *Pellaea* section *Ormopteris* are not especially close relatives. Previously sampled members of *Pellaea* section *Ormopteris* nested, albeit with poor support, within a large *Doryopteris* clade, sister to *Doryopteris* section *Lytoneuron* (Prado *et al.*, 2007). *Pellaea viridis* was resolved (support values were not reported) in a separate clade of primarily African species variously assigned to *Cheilanthes* and *Pellaea* (Eiserhardt *et al.*, 2011).

The morphological similarity of *Pellaea flavescens* and *P. viridis* is at odds with their presumed placement in separate, distantly related clades. However, *P. flavescens* itself has not been sampled in any previously published molecular phylogenetic study, leaving the question of its evolutionary position unanswered. Here, we examine the relationships of this species, and the taxonomic implications thereof, through an analysis of plastid *rbcL* sequences.

Materials and Methods

A plastid *rbcL* sequence was obtained from a recent collection of *Pellaea flavescens* following established protocols (Schuettpelz and Pryer, 2007). A dataset was then assembled, consisting of this sequence, 22 exemplars from clade D of Eiserhardt *et al.* (2011) obtained from GenBank, and an additional published sequence of *P. viridis* (Table 1). These sequences were manually aligned using Mesquite version 2.75 (Maddison and Maddison, 2011) and subsequently analyzed using MRBAYES version 3.2.1 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003). Regions at the ends of the alignment containing copious amounts of missing data were excluded, leaving a dataset of 1143 characters to which a GTR+G model of sequence evolution was assigned. Our Bayesian analysis comprised two independent runs, each utilizing four chains of two million generations. Trees were sampled every 1000 generations. To identify when the runs had reached stationarity, the standard deviation of the split frequencies between the two runs was examined, and the output parameter estimates were plotted using Tracer 1.5 (Rambaut and Drummond, 2009). Based on these convergence diagnostics, the first 500 trees were (very conservatively) excluded from each analysis before obtaining a majority rule consensus phylogeny with clade posterior probabilities. The resulting tree was rooted with *Trachypteris pinnata* (Hook. f.) C. Chr.

Results and Discussion

The *rbcL* sequence obtained from our Brazilian *Pellaea flavescens* sample was identical to two Old World exemplars of *P. viridis* (EF452147 and GU935494; Table 1). These sequences compose a well-supported (posterior probability, PP = 1.00) clade in our recovered phylogeny (Fig. 1) that is, in turn, robustly supported (PP = 1.00) as sister to a third exemplar of *P. viridis*. *Pellaea* section *Ormopteris*, here represented by *P. gleichenioides* (Gardn.) Christ and *P. pinnata* (Kaulf.) Prantl, is not closely related to this clade. Instead, it is well-supported as sister to *Doryopteris* section *Lytoneuron* (PP = 1.00), consistent with the findings of Prado *et al.* (2007). Thus, the placement of *P. flavescens* in *Pellaea* section *Ormopteris* by Tryon and Tryon (1982) is not supported by our molecular analysis.

The phylogenetic position of *Pellaea flavescens* as closely related to *P. viridis*, is supported by morphology. In fact, these taxa were recognized as a "species pair" by Moran and Smith (2001), who noted only slight structural differences. Here, however, we find *P. flavescens* to be nested within *P. viridis*, having an *rbcL* sequence identical to those of two *P. viridis* individuals (Fig. 1). Although *rbcL* is among the more slowly evolving plastid genes, a recent study of candidate barcodes (Li *et al.*, 2011) found it to be comparable to a much more rapidly evolving gene (*matK*) in its discriminatory power. Upwards of 40% of the species in the *Cheilanthes marginata* group (now recognized as the genus *Gaga*; Li *et al.*, 2012) could be separated based on *rbcL* alone (Li *et al.*, 2011). Unfortunately, but not surprisingly, discriminatory power was weakest

TABLE 1. List of sampled taxa, including GenBank accession numbers, source localities, and voucher information.

Species	Accession	Locality	Voucher information
Adiantopsis chlorophylla (Sm.) Fée	EF473684	Brazil	Prado *et al.* (2007)
Adiantopsis radiata (L.) Fée	EF452131	Guadeloupe	Schuettpelz *et al.* (2007)
Cheilanthes flexuosa Kunze	EF473686	Brazil	Prado *et al.* (2007)
Cheilanthes goyazensis (Taub.) Domin	EF473687	Brazil	Prado *et al.* (2007)
Cheilanthes induta Kunze	GU935501	South Africa	Eiserhardt *et al.* (2011)
Cheilanthes multifida (Sw.) Sw.	GU935499	South Africa	Eiserhardt *et al.* (2011)
Cheilanthes namaquensis (Baker) Schelpe & N.C. Anthony	GU935492	South Africa	Eiserhardt *et al.* (2011)
Cheilanthes quadripinnata (Forssk.) Kuhn	GU935496	South Africa	Eiserhardt *et al.* (2011)
Cheilanthes venusta Fée	JN122014	Brazil	Eiserhardt *et al.* (2011)
Doryopteris collina (Raddi) J. Sm.	EF473688	Brazil	Prado *et al.* (2007)
Doryopteris concolor (Langsd. & Fisch.) Kuhn	U05621	Taiwan	Hasebe *et al.* (1994)
Doryopteris lomariacea Klotzsch	EF473689	Brazil	Prado *et al.* (2007)
Doryopteris ornithopus (Hook. & Baker) J. Sm.	EF473691	Brazil	Prado *et al.* (2007)
Doryopteris pedata (L.) Fée	U27206	Mexico	Gastony and Rollo (1995)
Doryopteris pentagona Pic. Serm.	EF473693	Brazil	Prado *et al.* (2007)
Pellaea calomelanos (Sw.) Link	GU935497	Namibia	Eiserhardt *et al.* (2011)
Pellaea flavescens Fée	JX455163	Brazil	Prado 2036 (SP)
Pellaea gleichenioides (Gardn.) Christ	EF473698	Brazil	Prado *et al.* (2007)
Pellaea pinnata (Kaulf.) Prantl	EF473699	Brazil	Prado *et al.* (2007)
Pellaea pteroides (L.) Prantl	GU935502	South Africa	Eiserhardt *et al.* (2011)
Pellaea viridis (Forssk.) Prantl	EF452147	Réunion	Schuettpelz *et al.* (2007)
Pellaea viridis (Forssk.) Prantl	GU935494	South Africa	Eiserhardt *et al.* (2011)
Pellaea viridis (Forssk.) Prantl	GU935495	South Africa	Eiserhardt *et al.* (2011)
Trachypteris pinnata (Hook. f.) C. Chr.	U27450	Bolivia	Gastony and Rollo (1995)

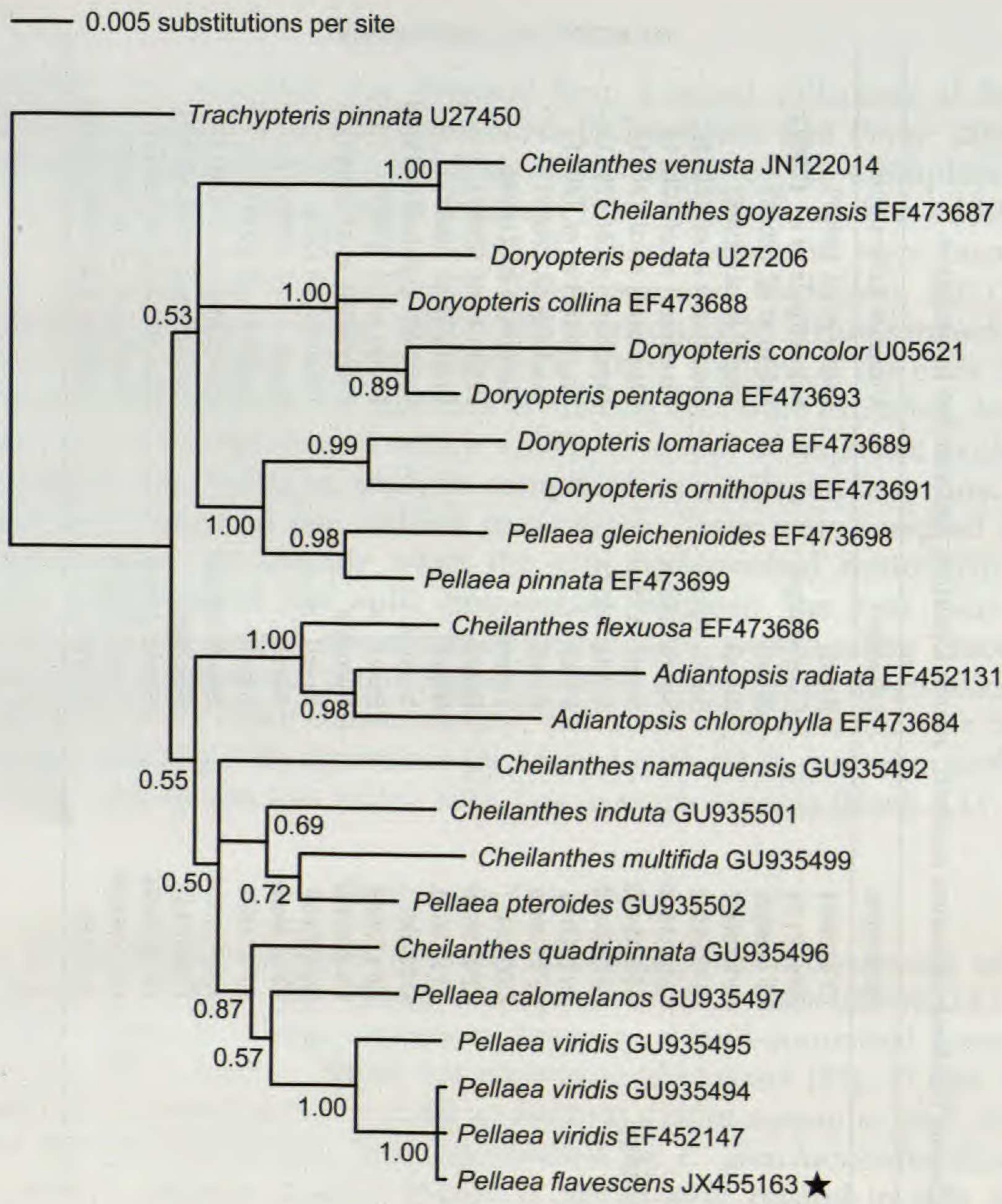

FIG. 1. Phylogeny resulting from Bayesian analysis of plastid *rbcL* sequences. Numbers at nodes are posterior probabilities. Brazilian *Pellaea flavescens*, here recognized as a synonym of Old World *P. viridis*, is marked with a star.

within species complexes. Nonetheless, based on the available evidence, and pending more exhaustive sequencing and a detailed study of the *Pellaea viridis* complex (see below), we recommend treating *P. flavescens* and *P. viridis* as synonyms, with the latter having priority.

The members of what is herein referred to as the *Pellaea viridis* complex will eventually need to be accommodated in some genus other than *Pellaea* or *Cheilanthes*, as the types of these genera are resolved elsewhere in the cheilanthoid phylogeny (Eiserhardt *et al.*, 2011; Kirkpatrick, 2007). Another point of nomenclatural clarification concerns *P. bongardiana* Baker, the description of which cited the type of *P. flavescens*. The former name is

therefore superfluous and cannot be used unless conserved or sanctioned, according to the International Code of Nomenclature for algae, fungi, and plants (McNeill *et al.*, 2012). In any case, the figure cited for *P. bongardiana* in the *Flora Brasiliensis* (tab. 55, fig. II) is wrong, with the illustrated plant representing *Pellaea riedelii* Baker.

Pellaea viridis is commonly cultivated (Hoshizaki and Moran, 2001), and escape from cultivation, followed by naturalization, has been documented (e.g., in Hawaii, by Palmer, 2003; in eastern Australia, by Bostock, 1998; and in Florida, see Atlas of Florida vascular plants: http://florida.plantatlas.usf.edu/). Thus, it may be that the existence of this species in Brazil represents yet another introduction from cultivation into the wild. Despite this apparently reasonable possibility, *P. viridis* is not currently cultivated in Brazil, so far as we are aware, and the Brazilian populations generally occur in undisturbed environments. Furthermore, while the first Brazilian collection (*Glaziou 2473*) we have seen dates from 1867, the current distribution of the species in Brazil, nearly 150 years later, remains highly restricted (Prado and Hirai, 2011). This stands in stark contrast to what has been encountered for most other introduced species. For example, despite a much more recent introduction into the New World of the Old World fern *Thelypteris dentata* (Forssk.) E. P. St. John (the earliest New World collection dates from 1908; Strother and Smith, 1970), this species is today one of the most common ferns in many areas of the Neotropics (Mickel and Smith, 2004). Although the distribution of the apparently introduced *Marsilea hirsuta* in the Azores is even more restricted than that of *P. flavescens* in Brazil (Schaefer *et al.*, 2011), the absence of *Marsilea* in historical collections from the Azores would point to a very recent introduction of the genus there. It is possible, then, that the populations of *P. viridis* in Brazil are native, originating via long distance spore dispersal from the Old World. Although uncommon, such a disjunction is not unheard of: Moran and Smith (2001) documented 27 pteridophyte species that naturally occur in both the Neotropics and Africa.

Moving forward, it is clear that the *Pellaea viridis* complex is in need of further examination (Moran and Smith, 2001; Eiserhardt *et al.*, 2011), not only to clarify species boundaries but also to identify the affinities of the Brazilian and other New World collections. Only through a more thorough analysis will it be possible to uncover the true origin of this lineage in Brazil.

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LITERATURE CITED

ATLAS OF FLORIDA VASCULAR PLANTS. http://florida.plantatlas.usf.edu/ Accessed 25 July 2012.

BOSTOCK, P. D. 1998. *Pellaea*. Pp. 266–269 *in* Flora of Australia, v. 48. ABRS/CSIRO, Melbourne.

EISERHARDT, W. L., J. G. ROHWER, S. J. RUSSELL, J. C. YESILYURT and H. SCHNEIDER. 2011. Evidence for radiations of cheilanthoid ferns in the Greater Cape Floristic Region. Taxon 60:1269–1283.

GASTONY, G. and D. R. ROLLO. 1995. Phylogeny and generic circumscriptions of cheilanthoid ferns (Pteridaceae: Cheilanthoideae) inferred from *rbcL* nucleotide sequences. Amer. Fern J. 85:341–360.

HASEBE, M., T. OMORI, M. NAKAZAWA, T. SANO, M. KATO and K. IWATSUKI. 1994. *rbcL* gene sequences provide evidence for the evolutionary lineages of leptosporangiate ferns. Proc. Natl. Acad. Sci. U.S.A. 91:5730–5734.

HOSHIZAKI, B. J. and R. C. MORAN. 2001. Fern grower's manual. Revised and expanded edition. Timber Press, Portland, Oregon.

HUELSENBECK, J. P. and F. RONQUIST. 2001. MRBAYES: Bayesian inference of phylogeny. Bioinformatics 17:754–755.

KIRKPATRICK, R. E. B. 2007. Investigating the monophyly of *Pellaea* (Pteridaceae) in the context of a phylogenetic analysis of cheilanthoid ferns. Syst. Bot. 32:504–518.

LI, F.-W., L.-Y. KUO, C. J. ROTHFELS, A. EBIHARA, W.-L. CHIOU, M. D. WINDHAM and K. M. PRYER. 2011. *rbcL* and *matK* earn two thumbs up as the core DNA barcode for ferns. PLoS ONE 6:e26597.

LI, F.-W., K. M. PRYER and M. D. WINDHAM. 2012. *Gaga*, a new fern genus segregated from *Cheilanthes* (Pteridaceae). Syst. Bot. 37:845–860.

MADDISON, W. P. and D. R. MADDISON. 2011. Mesquite: A Modular System for Evolutionary Analysis. Version 2.75 http://mesquiteproject.org

MCNEILL, J., F. R. BARRIE, W. R. BUCK, V. DEMOULIN, W. GREUTER, D. L. HAWKSWORTH, P. S. HERENDEEN, S. KNAPP, K. MARHOLD, J. PRADO, W. F. PRUD'HOMME VAN REINE, G. F. SMITH, J. H. WIERSEMA and N. J. TURLAND (EDS.) 2012. International Code of Nomenclature for algae, fungi, and plants (Melbourne Code) adopted by the Eighteenth International Botanical Congress Melbourne, Australia, July 2011. Regnum Veg. 154:1–240.

MICKEL, J. T. and A. R. SMITH. 2004. The Pteridophytes of Mexico. Mem. New York Bot. Gard. 88:1–1055.

MORAN, R. C. and A. R. SMITH. 2001. Phytogeographic relationships between Neotropical and African-Madagascan pteridophytes. Brittonia 53:304–351.

PALMER, D. D. 2003. Hawai'i's ferns and fern allies. University of Hawai'i Press, Honolulu.

PRADO, J. and R. Y. HIRAI. 2011. *Pellaea flavescens* Fée in Rio de Janeiro, its lectotypification, and its new record for São Paulo State, Brazil. Amer. Fern J. 101:50–52.

PRADO, J., C. D. N. RODRIGUES, A. SALATINO and M. L. F. SALATINO. 2007. Phylogenetic relationships among Pteridaceae, including Brazilian species, inferred from *rbcL* sequences. Taxon 56:355–368.

RAMBAUT, A. and A. J. DRUMMOND. 2009. Tracer. Version 1.5 http://tree.bio.ed.ac.uk/software/tracer/

RONQUIST, F. and J. P. HUELSENBECK. 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574.

SCHAEFER, H., M. A. CARINE and F. J. RUMSEY. 2011. From European priority species to invasive weed: *Marsilea azorica* (Marsileaceae) is a misidentified alien. Syst. Bot. 36:845–853.

SCHUETTPELZ, E. and K. M. PRYER. 2007. Fern phylogeny inferred from 400 leptosporangiate species and three plastid genes. Taxon 56:1037–1050.

SCHUETTPELZ, E., H. SCHNEIDER, L. HUIET, M. D. WINDHAM and K. M. PRYER. 2007. A molecular phylogeny of the fern family Pteridaceae: Assessing overall relationships and the affinities of previously unsampled genera. Molec. Phylogenet. Evol. 44:1172–1185.

STROTHER, J. L. and A. R. SMITH. 1970. Chorology, collection dates, and taxonomic responsibility. Taxon 19:871–874.

TRYON, R. M. and A. F. TRYON. 1982. Ferns and allied plants with special reference to tropical America. Springer-Verlag, New York.